Clinical Applications of MRI Methods that Assess Tumor Vascular Function

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Several commonly available imaging techniques are able to assess human tumors with respect to their angiogenic status. Both CT and MRI have the advantage of good spatial resolution which is often equal to that of corresponding morphological images. They are minimally invasive, involve little patient risk and data acquisition is quick thus allowing their incorporation into routine patient studies. MRI techniques are also sensitive to a variety of contrast mechanisms including blood flow, microvessel permeability and diameter, tissue oxygenation and water diffusion. The clinical application of MRI techniques will be the focus of this lecture.

Perfusion Imaging with Exogenous Contrast Agents

Currently the only available contrast agents for human perfusion imaging are 'low molecular weight' agents (typically <1kDa) which diffuse freely between the intravascular and extravascular, extracellular space (EES) but never cross cell membranes. These contrast agents have a high first pass extraction in most normal (with the exception of the brain, testes and retina) and tumor tissues. Three major factors determine the behaviour of low molecular weight contrast media in tissues during the first few minutes after injection: blood perfusion, transport of contrast agent across vessel walls and diffusion of contrast medium in the interstitial space. If the delivery of the contrast medium to a tissue is insufficient (flow-limited situations or where vascular permeability is greater than inflow) then blood perfusion will be the dominant factor determining contrast agent kinetics; this situation is commonly found in tumors. If tissue perfusion is sufficient and transport out of the vasculature does not deplete intravascular contrast medium concentration (non-flow limited situations – e.g. in areas of fibrosis or after treatment) then transport across the vessel wall is the major factor that determines contrast medium kinetics. As low molecular weight contrast media do not cross cell membranes, their volume of distribution is effectively the interstitial space. It is the differences in these contrast agent kinetics between normal tissue and tumor that are exploited by both and dynamic MRI to provide lesion/tissue specific information.

When injected as a paramagnetic bolus, gadolinium containing MRI contrast agents are transiently confined within the vascular space. While in that vascular space they produce magnetic field (Bo) inhomogenities that result in a decrease in the signal intensity of surrounding tissues. MR sequences can be designed to be sensitive to the vascular phase of contrast medium delivery (so-called T_2* or susceptibility-based methods which reflect on tissue perfusion and blood volume). Similarly, sequences sensitive to the presence of contrast medium in the EES reflect on microvessel perfusion, permeability and extracellular leakage space (so-called T_1 or relaxivity based methods). These methods are compared with f-MDCT in the table.

Comparison of dynamic-MRI with functional-MDCT

	Dynamic susceptibility contrast enhanced MRI (DSC-MRI)	**Dynamic relaxivity contrast enhanced MRI (DCE-MRI)**
Mechanism of tissue enhancement	Susceptibility effects of contrast agent on magnetic field	Relaxivity effects of contrast agent on tissue water
Tissue compartment being interrogated	Vascular space	Vascular and extravascular space
Tissue signal intensity change	Darkening	Enhancement
Duration of effect and optimal data acquisition	Seconds / every 1-2 seconds	Minutes / 2-25 seconds

Magnitude of effect	Small	larger
SNR	Low	Very high
Quantification method used	Central volume theorem	General multi-compartment pharmacokinetic model
Kinetic parameters measured	Relative Blood Flow, Relative Blood Volume, Mean Transit Time	Transfer constants, leakage space, blood volume and flow

1. Dynamic susceptibility contrast enhanced MRI (DSC-MRI)

Perfusion-weighted images can be obtained with "bolus-tracking techniques" that monitor the passage of contrast material through a capillary bed (Sorensen, Tievsky et al. 1997; Barbier, Lamalle et al. 2001). The decrease in signal intensity of tissues can be observed with susceptibility-weighted T_1 or T_2*-weighted sequences, the latter providing greater sensitivity and contrast to perfusion effects. In this context, spin-echo sequences are more sensitive to capillary blood flow compared with gradient-echo sequences, which incorporate signals from larger vessels (Simonsen, Ostergaard et al. 2000). The degree of signal loss observed is dependent on the vascular concentration of the contrast agent and microvessel size (Dennie, Mandeville et al. 1998) and density. The signal to noise ratio (SNR) of such images can be improved by using high doses of contrast medium (i.e. ≥ 0.2-mmol/kg body weight) (Bruening, Berchtenbreiter et al. 2000). High specification echo-planar capable MRI systems which allow rapid image acquisition are required to adequately characterize these effects. Such studies are possible on conventional MRI systems using standard gradient-echo sequences but are limited to fewer slices.

Tracer kinetic principles can be used to provide estimates of relative blood volume (rBV), relative blood flow (rBF) and mean transit time (MTT) derived from the first-pass of contrast agent through the microcirculation (Rosen, Belliveau et al. 1991; Sorensen, Tievsky et al. 1997; Barbier, Lamalle et al. 2001). These variables are related by the central volume theorem equation (BF = BV/MTT). The most robust parameter that can be derived from the first pass is rBV; this is obtained from the integral of the time series data of the first pass (Ostergaard, Smith et al. 1998). For extracranial tumors, the time series data is usually fitted to a gamma variate function from which kinetic parameters are obtained. Absolute quantification is not currently possible for the evaluation of visceral tissues and tumors. From a practical perspective, it is not always necessary to quantify T_2*-weighted DSC-MRI data to obtain insights of the relative distribution of tissue perfusion. Simple subtraction images can be calculated to demonstrate the maximal signal drop which in turn has been strongly correlated with relative blood flow and volume in tumors (Cha, Lu et al. 2000; Liu, Chung et al. 2002).

Quantitative DSC-MRI is currently most reliable in brain applications as the contrast medium is largely retained within the intravascular space (Knopp, Cha et al. 1999). There is very little data in the literature regarding the use of DSC-MRI outside the brain. Qualitative observations of signal loss observed on DSC-MRI have been reported in preliminary clinical studies to characterize liver, breast and brain lesions. For example, Ichikawa et al. were able to discriminate between liver metastases, hemangiomas and hepatomas on the basis of characteristic signal intensity changes on echo-planar MRI (Ichikawa, Haradome et al. 1998). Both Kuhl et al. and Kvistad et al. have qualitatively evaluated the value of DSC-MRI for characterising breast lesions (Kuhl, Bieling et al. 1997; Kvistad, Lundgren et al. 1999). Both studies showed strong signal intensity decreases in malignant tissues with only minor susceptibility effects in fibroadenomas.

2. Dynamic relaxivity contrast enhanced MRI (DCE-MRI)

Most dynamic relaxivity enhanced DCE-MRI studies employ T_1W gradient-echo sequences to monitor the tissue enhancing effects of contrast media. This is because gradient-echo sequences have good contrast medium sensitivity, high signal to noise ratio and the data acquisition can be performed rapidly. Unlike f-MDCT, the degree of signal enhancement seen on T_1-weightedMRI is dependent on a number of physiological and physical factors. These include tissue perfusion, capillary permeability to contrast agent, extracellular leakage space volume, native T_1-relaxation rates of the

tissue, contrast agent dose (and its protein binding), imaging sequence used, imaging parameters utilised and on machine scaling factors.

Signal enhancement seen on a dynamic acquisition of T_1-weighted images can be assessed either by analysing signal intensity changes (semi-quantitative) and/or by quantifying contrast agent concentration changes using pharmacokinetic modelling techniques (Figure 3). Semi-quantitative parameters have the advantage of being relatively straightforward to calculate but have a number of limitations including an inability to accurately reflect contrast medium concentration in the tissue of interest. They can also be influenced by scanner settings. Quantitative methods use pharmacokinetic modelling techniques that are applied to tissue contrast agent concentration changes. Signal intensity changes during dynamic acquisition are used to estimate contrast agent concentration *in vivo* (Parker, Suckling et al. 1997; Parker, Baustert et al. 2000). Quantitative parameters are more complicated to derive which deters their use at the workbench. The main advantage of quantification is the ability to directly compare examinations acquired serially in a given patient and in different patients imaged at the same or different scanning sites.

Many studies have attempted to correlated tissue MR enhancement with immuno-histochemical microvessel density (MVD) measurements. Some MRI studies have shown broad correlations between T_1 kinetic parameters estimates and MVD whereas others have not. Recently, VEGF which as noted above is a potent vascular permeability and angiogenic factor has been implicated as an additional explanatory factor that determines MR signal enhancement although the relationship between MRI enhancement and tissue VEGF expression is not straightforward. Other characteristics that have been correlated with enhancement patterns include the degree of stromal cellularity and fibrosis and tissue oxygenation (see Padhani and Dzik-Jurasz (Padhani and Dzik-Jurasz 2004) for a comprehensive review).

Enhancement seen on T_1-weighted DCE-MRI is a valuable tool in a number of clinical situations. The most established role is in lesion characterization where it has found a role in distinguishing benign from malignant breast and musculoskeletal lesions (Kaiser and Zeitler 1989; van der Woude, Verstraete et al. 1998). Dynamic T_1-weighted MRI studies have also been found to be of value in staging gynaecological malignancies, bladder and prostate cancers (Huch Boni, Boner et al. 1995; Barentsz, Jager et al. 1996; Jager, Ruijter et al. 1997; Liu, Krestin et al. 1998). DCE-MRI studies have also been found to be of value in detecting tumor relapse in the presence of fibrosis within treated tissues of the breast and pelvis (Dao, Rahmouni et al. 1993; Gilles, Guinebretiere et al. 1993; Heywang-Kobrunner, Schlegel et al. 1993; Kerslake, Fox et al. 1994; Mussurakis, Buckley et al. 1995; Kinkel, Tardivon et al. 1996; Blomqvist, Fransson et al. 1998; Hawnaur, Zhu et al. 1998). Recently, DCE-MRI has been shown to be of value for screening women at high genetic risk of breast cancer (Leach, Boggis et al. 2005). DCE-MRI is also able to predict response or monitor the effects of a variety of treatments. These include neoadjuvant chemotherapy in bladder and breast cancers and bone sarcomas (Knopp, Brix et al. 1994; van der Woude, Bloem et al. 1995; Barentsz, Berger-Hartog et al. 1998; Reddick, Taylor et al. 1999; Padhani, MacVicar et al. 2001). Other treatments that can be monitored include radiotherapy in rectal and cervix cancers (de Vries, Griebel et al. 2000; Mayr, Yuh et al. 2000; Devries, Griebel et al. 2001; George, Dzik-Jurasz et al. 2001) and androgen deprivation in prostate cancer (Padhani, MacVicar et al. 2001). A number of studies have recently reported on the use of T_1-weighted DCE-MRI for monitoring the effects of antiangiogenic/antivascular treatments (Galbraith, Maxwell et al. 2003; Morgan, Thomas et al. 2003). These response assessment studies show that successful treatment results in a decrease in the rate and magnitude of enhancement and that poor response results in persistent abnormal enhancement.

3. MRI with macromolecular weight contrast media (MMCM)

ECF contrast agents have a high first pass extraction fraction in both normal and abnormal tissues (Daldrup, Shames et al. 1998). MMCM have molecular sizes that approximate some serum proteins and have minimal first pass extraction fraction in normal vessels and therefore appear well suited for the measurement of tumor macromolecular hyperpermeability (Daldrup, Shames et al. 1998; Roberts, Roberts et al. 1998; Su, Muhler et al. 1998). MMCM are probably delivered to the interstitial space by non-specific vesicular transport (vesiculo-vacuolar organelles) or through transendothelial channels (Dvorak, MacGlashan et al. 1996). Only preclinical validation of MMCM

techniques appears in the literature but approval of agents for human use is expected soon. Albumin-$(Gd\text{-}DTPA)_{30}$ is the prototype MMCM (70-90 kDa) but this agent has been found to be immunogenic and has significant retention in the liver and bone (Schmiedl, Ogan et al. 1987). Polylysine-(Gd-DTPA) is not readily biodegradable which also makes it unsuitable for human use. Other Gadolinium based MMCM (e.g., Gadomer 17 and the macromolecular Gd-DOTA derivate P792) are currently in advanced clinical trials and licensing for human use for these agents is expected soon. Ultrasmall superparamagnetic iron oxide (USPIO) particles (diameter 20-30 nm) have been investigated as MMCM for the evaluation of angiogenesis (Turetschek, Huber et al. 2001; Turetschek, Roberts et al. 2001).

Imaging vascular function using haemoglobin as a contrast agent (Intrinsic Susceptibility MRI)

Analysis of vascular function can be accomplished by using deoxyhaemoglobin as an intrinsic, paramagnetic contrast agent (blood oxygenation level dependent or BOLD contrast; also called Intrinsic Susceptibility Contrast) (Howe, Robinson et al. 2001). Gradient-echo T_2* weighted images are used are used; the signal intensity seen on BOLD images is dependent on tissue structure, local blood flow and on the oxygenation status of hemoglobin. The relaxivity of tissues (R_2*=1/T_2*) can be quantified relativity easily using multi-gradient echo sequences with lengthening echo-times. Changes in BOLD signal in response to an exogenous stimulus is due to alteration in blood volume, blood flow and blood oxygenation. BOLD contrast can therefore be used for mapping changes in blood volume fraction, and vascular functionality associated with angiogenesis and anti-angiogenesis (Abramovitch, Dafni et al. 1999; Neeman, Dafni et al. 2001). Vascular function can be evaluated by analysis of BOLD contrast changes in response to hyperoxia and hypercapnia (Howe, Robinson et al. 2001). Clinical application of this technique has revealed high signal enhancements in response to carbogen (5% $C0_2$: 95%0_2) inhalation in human tumors (Taylor, Baddeley et al. 2001). Taylor *et al* have also reported that human studies are technical challenging (Taylor, Baddeley et al. 2001). The primary advantage of BOLD techniques is that there is no need to administer contrast material. Measurements can be repeated as needed with almost no limitation. BOLD contrast is not sensitive to fluctuation in permeability. A major reservation for intrinsic contrast imaging is the low contrast to noise ratio in the images obtained.

Imaging vascular function using water diffusion (Diffusion Weighted MRI; DW-MRI)

In basic terms DW-MRI looks at the random (Brownian) motion of water molecules and the factors that restrict or increase water mobility. In human tissues it is possible to assess the different contributions to the mobility of water molecules by applying to T2–weighted sequences additional diffusion-weighting. This entails the application of two extra opposing (or balanced) gradient of differing durations and amplitudes. For water molecules that show no net movement over time, the application balanced gradients results in no change in signal intensity in diffusion weighted images. Conversely, applying balanced gradients to water molecules with net movement will no longer cancel out, thus affecting the measured signal intensity. The degree of diffusion-weighting is termed the b-value (measured in sec mm^2), which depends on the magnitude of the gradient, duration of the gradient and time between the two gradient pulses (Schaefer PW, Grant PE et al. 2000). The apparent diffusion coefficient (ADC; the observed displacement of water molecules per unit time in mm^2/sec) of water in tissues is dependent on its microenvironment. Thus, DWI is not just sensitive to microscopic movements of water but will also be sensitive to other physiological motions of greater magnitude, such as blood and CSF flow which therefore make a significant contribution to the measured the measured ADC (Le Bihan D, Breton E et al. 1988; Turner R, Le Bihan D et al. 1990; Turner R, Le Bihan D et al. 1991). At lower b values (<100 sec/mm^2) tumor perfusion rather than extracellular water diffusion will be the predominant factor in determining the ADC of water (Le Bihan D, Breton E et al. 1988; Morvan D 1995; Thoeny HC, De Keyzer F et al. 2004). With increased diffusion-weighting (i.e. higher b-values) there will be increased filtering out of high mobility molecules and one so will be able to differentiate between high and low mobility water populations (Niendorf T, Dijkhuizen RM et al. 1996). Higher b-values result in filtering out perfusion changes and so the calculated ADC can become a true measure of the diffusion coefficient D. At high b-values, it is not entirely clear whether the measurement of the differing mobility populations truly represents the intracellular or extracellular components of the total water diffusion measured, which may not be

great significance (Mulkern RV, Vajapeyam S et al. 2005). Thus, the magnitude of the b-value used in DWI is an important consideration when assessing pathological or pharmacological processes that alter blood flow, especially when one may want to have a particular interest in diffusion changes rather than the vascular effects.

There has been a huge increase in the number of diffusion MRI publications within the past five years mostly in neurology. With regards to oncology, research has focused mainly on the diagnostic value of DWI (at high b-value) in assessing the presence and extent of tumors within organs (eg, prostate and breast) and in predicting and assessing early response to treatment. For example serial ADCs can be measured before and after the introduction of anti-neoplastic treatment, which then could be used as a pharmacodynamic indicator of drug activity when correlated with histological changes. The use of perfusion weighted DW-MRI using low b-values is relativity under explored in humans but the potential has been demonstrated. For example, Thoeny et al recently used DWI to evaluate the antivascular effects of the compound CA4P; which is currently undergoing clinical trials. They showed rapid antivascular effects in xenografts at 1 and 6 hours post CA4P when falls in ADC values were observed which histologically corresponded to vessel congestion and vascular shutdown, but no necrosis (Thoeny HC, De Keyzer F et al. 2005). A more recently published study by Thoeny assessed tumor response to multiple administrations of CA4P with similar results seen after each treatment (Thoeny HC, De Keyzer F et al. 2005). The results demonstrate the capability of DWI to distinguish not only changes in vascularity but also between viable and nonviable tissue due to changes in tumor cellularity (Lang P, Wendland MF et al. 1998; Lyng H, Haraldseth O et al. 2000).

Conclusions

There is a definite clinical need for non-invasive tumor angiogenesis imaging assays. Ultrasound with microbubbles as contrast agents, perfusion CT, DSC-MRI and DCE-MRI are currently the favored techniques for evaluating tumors with respect to their functional microcirculation but encouraging data with other MRI techniques is beginning to appear. The choice between techniques used in the clinic will be determined by several key factors including local availability and expertise, tumor site, desired perfusion parameter and the need to reduce radiation burden. The widespread availability of CT may be a major determinant in future use. To date there have been no comprehensive studies that have compared the performance of functional CT and dynamic MRI. A number of challenges must be met if quantitative imaging of angiogenesis is to enter wider clinical practice. These include the need for commercial equipment manufacturers to provide robust methods for rapidly measuring time-varying changes in tissue contrast agent concentration and robust analysis software with validated statistical tools for the evaluation of heterogeneity. Such developments will be essential for multicenter trials where it will be necessary to establish effective cross-site standardization of measurements and evaluation. As imaging scientists and clinicians, the radiological community will need to become enthusiastic key players if there is to be successful clinical implementation of angiogenesis imaging.

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